

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 3 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] -- September 30, 2009

Listing of Claims:

Claims 1-111 (Canceled).

112. (Currently Amended) An *in vitro* process for producing more than one copy of a DNA molecule of interest, said process comprising the steps of:

- (a) providing a nucleic acid sample containing said DNA molecule of interest;
- (b) contacting said sample with a mixture comprising: (i) nucleic acid precursors; (ii) one or more specific polynucleotide primers comprising at least one ribonucleic acid segment, each of which primer comprises a sequence complementary to a distinct sequence of said DNA molecule of interest; (iii) an effective amount of a nucleic acid producing catalyst; and (iv) RNase H;
- (c) carrying out nucleic acid synthesis to extend a primer bound to said DNA molecule of interest and produce a polynucleotide comprising an RNA/DNA hybrid, thereby generating a substrate for RNase H; and
- (d) digesting said substrate with RNase H to remove said ribonucleic acid segment of said extended primer ~~and allow~~ , **wherein said removal allows** another primer binding event to occur with said ~~nucleic acid~~ **DNA molecule** of interest, thereby producing multiple copies of said nucleic acids of interest.

113. (Previously Presented) The process of claim 112, wherein said primers (ii) comprise modified nucleotides, unmodified nucleotides or a combination thereof.

114. (Previously Presented) The process of claim 112, wherein said primers (ii) comprise sequences noncomplementary to said distinct sequence of said DNA molecule of interest.

115. (Previously Presented) The process of claim 114, wherein said primers (ii)

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 4 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] --- September 30, 2009

comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

116. (Previously Presented) The process of claim 112, wherein said primers (ii) further comprise deoxyribonucleotides.

117. (Previously Presented) The process of claim 112, wherein said nucleic acid producing catalysts (iii) comprise DNA polymerase, reverse transcriptase or a combination thereof.

118. (Previously Presented) The process of claim 117, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria or a combination thereof.

119. (Previously Presented) The process of claim 118, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

120. (Previously Presented) The process of claim 112, wherein said mixture recited in step (b) comprises labeled nucleic acid precursors, one or more specific labeled polynucleotide primers, or a combination of both.

Claims 121-122 (Canceled).

123. (Previously Presented) A process for multiply initiating polynucleotide or oligonucleotide synthesis of a DNA molecule of interest comprising:

- (a) providing a sample containing said DNA molecule of interest;
- (b) contacting said sample with a mixture comprising: (i) nucleic acid precursors; (ii) one or more specific copolymer primers comprising at least one DNA segment and at least one RNA segment, each of which primer comprises a sequence

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 5 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] — September 30, 2009

complementary to a distinct sequence of said DNA molecule of interest; (iii) an effective amount of a nucleic acid producing catalyst; and (iv) RNase H;

(c) producing at least one copy of said DNA molecule of interest by using said nucleic acid producing catalyst (iii) and said DNA molecules of interest as templates to extend said copolymer primer; and

(d) removing said RNA segment of said extended copolymer primer from said template by digesting with RNase H to bind another copolymer primer to said template and initiate synthesis, thereby multiply initiating polynucleotide or oligonucleotide synthesis.

124. (Previously Presented) The process of claim 123, wherein said primers comprise modified nucleotides, unmodified nucleotides or a combination thereof.

125. (Previously Presented) The process of claim 123, wherein said primers further comprise sequences that are noncomplementary to said DNA molecule of interest.

126. (Previously Presented) The process of claim 125, wherein said primers comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

127. (Previously Presented) The process of claim 123, wherein the nucleic acid producing catalyst (iii) comprises DNA polymerase, reverse transcriptase or a combination thereof.

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 6 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] — September 30, 2009

128. (Previously Presented) The process of claim 127, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria or a combination thereof.

129. (Previously Presented) The process of claim 128, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

130. (Previously Presented) The process of claim 123, wherein said mixture recited in step (b) comprises labeled nucleic acid precursors, one or more specific labeled polynucleotide primers or a combination of both.

Claims 131-132 (Canceled).

133. (Previously Presented) An *in vitro* process for producing more than one complementary copy of an RNA molecule of interest, said process comprising the steps of:

- (a) providing a nucleic acid sample containing said RNA molecule of interest;
- (b) contacting said sample containing with a mixture comprising: (i) nucleic acid precursors; (ii) one or more polynucleotide primers wherein said primers comprise (A) at least one ribonucleic acid segment and (B) a sequence complementary to a distinct sequence in said RNA molecule of interest; (iii) an effective amount of a nucleic acid producing catalyst; and (iv) RNase H;
- (c) producing a first DNA copy from said RNA molecule of interest, by binding said polynucleotide primer with said RNA molecule of interest as a template;
- (d) using said first DNA copy as a template to produce a double-stranded nucleic acid comprising a second copy complementary to said DNA copy produced in step (c); and
- (e) removing said ribonucleic acid segment of said extended primers with

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 7 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] -- September 30, 2009

RNase H from said first DNA double-stranded copy produced in step (d) to generate a primer binding site on said second copy of (c) to render said primer binding site available for subsequent primer binding and extension events and producing more than one copy of said RNA molecule of interest.

134. (Previously Presented) The process of claim 133, wherein said primers (ii) comprise modified nucleotides, unmodified nucleotides or a combination thereof.

135. (Previously Presented) The process of claim 133, wherein said primers (ii) further comprise sequences noncomplementary to said distinct sequence of said RNA of interest.

136. (Previously Presented) The process of claim 135, wherein said primers (ii) further comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

137. (Previously Presented) The process of claim 133, wherein said primers (ii) further comprise deoxyribonucleotides.

138. (Previously Presented) The process of claim 133, wherein said nucleic acid producing catalysts (iii) comprise DNA polymerase, reverse transcriptase or a combination thereof.

139. (Previously Presented) The process of claim 133, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria, or a combination thereof.

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 8 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] -- September 30, 2009

140. (Previously Presented) The process of claim 133, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

Claims 141-142 (Canceled).

143. (Currently Amended) An in vitro process for producing more than one copy of a DNA molecule, said process comprising the steps of:

- (a) providing a nucleic acid sample containing or suspected of containing said DNA molecule;
- (b) contacting said sample with a mixture comprising:
 - (i) nucleic acid precursors,
 - (ii) specific polynucleotide primers comprising at least one ribonucleic acid segment, each of which primer is substantially complementary to a distinct sequence of said DNA molecule,
 - (iii) an effective amount of a DNA polymerase; and
 - (iv) an effective amount of RNase H;
- (c) allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said DNA molecule by extension of said primer; and
- (d) removing ribonucleotides from said ribonucleic acid segment using said RNase H, to regenerate a primer binding site on said DNA molecule, to render said primer binding site available for another primer binding event and thereby producing more than one copy of said DNA molecule.

144. (Previously Presented) The method of claim 143, wherein said primers are DNA/RNA copolymers which comprise said RNA segment, and further comprise a DNA segment.

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 9 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] — September 30, 2009

145. (Previously Presented) The method of claim 143, wherein said primers further comprise sequences which are non-complementary to said DNA molecule.

146. (Currently Amended) An in vitro process for producing more than one copy of a DNA molecule, said process comprising the steps of:

(a) providing a nucleic acid sample containing or suspected of containing said DNA molecule;

(b) contacting said sample with a mixture comprising:

(i) nucleic acid precursors,

(ii) specific polynucleotide primers comprising at least one ribonucleic acid segment, each of which primer is substantially complementary to a distinct sequence of said DNA molecule, and

(iii) an effective amount of a reverse transcriptase having RNase H activity;

(c) allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said DNA molecule by extension of said primer; and

(d) removing ribonucleotides from said ribonucleic acid segment using said reverse transcriptase, to regenerate a primer binding site on said DNA molecule, to render said primer binding site available for another primer binding event and thereby producing more than one copy of said DNA molecule.

147. (Previously Presented) The method of claim 146, wherein said primers are DNA/RNA copolymers which comprise said RNA segment, and further comprise a DNA segment.

148. (Previously Presented) The method of claim 146, wherein said primers further comprise sequences which are non-complementary to said DNA molecule.

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.

Serial No.: 10/713,183

Filed: November 14, 2003

Page 10 [Amendment Under 37 C.F.R. §1.115 (In Reply To The April 1, 2009
Office Communication)] — September 30, 2009

149. (New) The process of claim 143, wherein said mixture recited in step (b) comprises labeled nucleic acid precursors, one or more specific labeled polynucleotide primers or a combination of both.

150. (New) The process of claim 149, wherein said polynucleotide primers comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

Enz-52(D2)(C)(D1)